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Diffusion of Water - Soluble Spin Labels in the
Aqueous Phase of Mammalian Cells

by

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SUMMARY

The objective of this study was to examine diffusion of small water soluble compounds in mammalian cell cytoplasm using a spin labeling technique. The proposal was based on the idea that water soluble spin label compounds within a cell would be in a more restricted microenvironment than they would be in free solution. Internal cell barriers such as organelles, microfilaments and microtubules could all act to prevent spin label - spin label associations. Spin label interactions lead to changes in the spin label signal which can be monitored. The degree of change is indicative of the number of molecules interacting and thus diffusing in a given space. This translational motion can be used to calculate diffusion. Additionally, rotational motion of the spin label molecules can also be measured and used as a measure of the viscosity of the cytoplasm.

In the initial phase of the study spin labels were tested for cytotoxicity and for effects on cell growth. An appropriate compound, 2, 2, 5, 5,-tetramethyl-3-carboxypyrroline-N-oxyl (PCAOL), was selected. A nonpermeable quenching agent, NiCl_2 , was chosen to eliminate extracellular signal.

The second and third phases were done in parallel. In one series of experiments rotational and translational motion of spin labels was measured in model systems where viscosity and spacing of barriers were known. For example high concentrations of sucrose lead to slow diffusion of label. Polyacrylamide beads with different sizes of internal spaces cause physical compartmentalization of spin label. This data was used to interpret the data concerning the movements of labels in the cells.

The cells studied were a well-characterized fibroblast line, 3T3 and their SV40 virus transformed counterparts. Spin label at various concentrations was added to cells. Changes in quench of spin label signal with changes in spin label concentration showed the presence of internal cytoplasmic microbarriers. The pattern of diffusion was different in growing cells and resting cells. Transformed clones were different than their normal counterparts. Cytochalasin B, a compound causing dissociation of microfilaments, allowed spin label to move more freely in cells. Colcemid, which causes depolymerization of microtubules had no effect on the cells. The details of these studies are published (Keith, Mastro, and Snipes, W. 1979; Mastro, and Keith 1981).

B. Index of Technical Reports: not applicable

C. Index of Publications:

1. Keith, A. D., A. Mastro and W. Snipes. 1979. Diffusion relationships between cellular plasma membrane and cytoplasm. In Low Temperature Stress in Crop Plants (eds. J. Lyons and J. Raison) Academic Press, New York, pp. 437-451.
2. Mastro, A. M. and A. D. Keith. Spin label viscosity studies of mammalian cell cytoplasm. In The Transformed Cell (eds. I. Cameron and T. Poole) Academic Press. (1981).

D. Conclusions

Spin label probes are suitable for studying the physical state of mammalian cell cytoplasm. With such probes we have concluded that cytoplasmic microbarriers inhibit free movement of small water soluble compounds in cells. The inhibition can be both translational (diffusion) and rotational (viscosity) depending on the cells and their state of growth.

E. List of Major Accomplishments

1. Development of a spin label system to examine living mammalian cells.
2. Measurement of viscosity of small molecules in mammalian cell cytoplasm.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) A technique using small water soluble spin labeled molecules was developed to measure diffusion and viscosity in mammalian cell cytoplasm. The non- cytotoxic molecules freely entered the cells. The signal of extracellular material was quenched with a broadening agent, NiCl ₂ . The movement of the intracellular molecules, both rotational and translational, is determined by the cell cytoplasm microenvironment. This microenvironment consists of many diffusion barriers such as organelles, microfilaments and microtubules.		

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The movements of spin labels in model systems such as solutions of various viscosities or matrices of defined small spaces, were used to interpret the movement of these same molecules in cells. Both growing and quiescent 3T3 fibroblasts and their SV40 virus transformed counterparts were tested as were cells treated with drugs which can change the cell microenvironment. Changes in growth state and cytoplasmic environment caused either a more free or a more restricted state in the cytoplasm as detected by the motion of the spin label molecules.

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